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A Comparison of Possible Primers for the Determination of  
Gross Energy in Urine and High Moisture Materials <sup>1/</sup>

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The problem of finding a method for determining the energy values of high moisture materials has become of increased importance with the emphasis on digestible and net energy studies. In general, the method used has been that of bomb calorimetry on the oven dried material; however, oven drying results in a loss of volatile matter which leads to low energy values. Attempts to overcome this difficulty have led to indirect methods such as the calculation of the energy of urine from its carbon content as used by Kleiber (6), and Swift and French (7). Eriksson (3) has estimated the volatile loss of urine on drying by the loss of volatile nitrogen.

A more direct method of determining the energy content of wet materials is by burning them directly with the aid of a primer. Colovos (2) and Flatt (5) used ethanol to burn silage and/or feces, whereas Bratzler (1) used ethanol and benzoic acid as primers for fresh feces. Recently, Fenner and Archibald (4) proposed dimethylformamide as a primer for fresh feces.

In an attempt to burn fresh bovine urine, the following primers were investigated: a cellulose absorbing block with ethylene glycol or paraffin oil, direct addition of ethylene glycol, paraffin oil, methyl lactate, benzoic acid, dioxane, catechol dissolved in dioxane, resorcinol dissolved in dioxane, sucrose, anisaldehyde, cresyl methyl ether, diethanolamine, piperidine, pyridine, ethyl acetoacetate, benzoyl peroxide, methyl cello-solve, ethyl cellosolve, butyl cellosolve, dimethylformamide, and various combinations of these. The primers were added to urine in the ratios of 1 part primer to 1 part urine, and 1 part primer to 2 parts urine. Generally both primer and urine were pipetted into the capsule; however, because of the high volatility of some of the primers, they were sealed in a small glass bulb and the bulb was broken with a glass rod just before the bomb was sealed. Cupric oxide, selenium dioxide, copper salicylate, sodium bismuthate, and silver sulphate were examined for catalytic activity by adding 10 mg. of each to 1 gm. of primer, but no beneficial effect was detected. In an attempt to measure the degree of combustion, the unburned matter in the residue present in the capsule after ignition was oxidized with standard dichromate solution. It became apparent that complete combustion (as we measured it) was obtained with certain urines, while other urines did not burn completely. The two most effective primers seemed to

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be 10% catechol in dioxane, and pyridine, but even with these primers it was not possible to burn all urines. With the very high energy ratio of primer to sample (20:1 or greater), it was impossible to obtain good precision on those samples which burned.

Fenner and Archibald (4) suggested dimethylformamide as a suitable primer for fresh feces because of its low volatility during preliminary weighing and its slow rate of combustion. In addition, we felt that the efficacy of a primer for wet materials may also be related to its energy content and miscibility with water. Butyl cellosolve, (ethylene glycol, mono-butyl ether), is a relatively nonvolatile solvent, miscible with water and has a higher caloric content than N,N-dimethylformamide. A comparison of dimethylformamide and butyl cellosolve as primers for 2 silages of different moisture content, was made at varying rates of primer to sample. The energy content of each primer, as determined, was: dimethylformamide, 6310 cal., and butyl cellosolve, 7701 cal.

The corn silage (30.87% D.M.) and alfalfa silage (23.56% D. M.) were ground in a meat grinder with a plate containing 7/32 inch holes, and then mixed. Samples were taken immediately, sealed in glass jars and stored in a refrigerator. Dry matter samples were taken from the glass jars. A sample was weighed directly into the combustion capsule and the primer was added to the sample with an eye dropper as the capsule rested on the balance. Two samples were weighed out at a time, one being primed with dimethylformamide and the other with butyl cellosolve, both primers being added as nearly as possible at the same ratio. The ratio of primer to sample was progressively widened until incomplete combustion was obtained. Approximately 2.5 gm. samples of silage were used throughout the experiment.

### Results and Discussion

Combustion was obtained at a wider ratio with the higher energy primer as shown in Table I. For comparative purposes, data are included on a sample of feces and of urine. With materials containing less water, wider ratios of primer to sample can be tolerated. From Table II it can be seen that the maximum percentage of water in the mixtures that burned was never greater than approximately two-thirds. Mixtures of distilled water with certain urine primers such as pyridine and 10% catechol in dioxane would burn at about this ratio, but generally it was more difficult to obtain combustion with primer-urine mixtures than with primer-water mixtures. This may have been due to the presence of non-combustible inorganic materials present in the urine. In accordance with this, it is seen from the ratio of total calories to gms. of water in Table II, that the efficiency of the primers decreases with increasing water content of the sample.

From the data accumulated on sample to primer ratios showing complete combustion, it was possible to observe a relationship between the sample to primer ratio and the apparent caloric content of the sample. In order to measure this, regression equations were calculated according to Snedecor (8).

The slopes of the regression lines are given in Table III. Negative slopes were obtained in all cases and in 3 out of 4 cases were statistically significant. Consequently, variation in the sample primer ratio would appear to be contributing to the error in determining the caloric content of wet materials by use of a primer. Possible explanations for this effect would be: the value attributed to the primer may have been too low, or there may have been slight incomplete combustion which was unnoticed by ordinary examination.

### Summary and Conclusions

Using various primers and combinations, it was not possible to burn all urines nor was it possible to obtain satisfactory precision due to the unfavorable sample to primer energy ratio necessary to obtain combustion.

In a study of sample to primer ratios with silages of varying moisture content, the primers appeared to become less efficient with materials of higher moisture content. Butyl cellosolve with a higher caloric content per gm. than dimethylformamide, appeared to be more efficient.

A statistically significant relationship was found between the sample to primer ratio and the apparent caloric content of the sample.

### References

1. Bratzler, J. W., and Swift, R. W. A Comparison of Nitrogen and Energy Determinations on Fresh and Oven-Air Dried Cattle Feces. J. Dairy Sci., 42: 686. 1959.
2. Colovos, N. F., Keener, H. A., and Davis, H. A. Errors in Drying Silage and Feces for Protein and Energy Determinations. Improved Procedures. J. Dairy Sci., 40: 173, 1957.
3. Eriksson, Sture. Determination of Energy in Urine. Reprinted from the Annals of the Royal Agricultural College of Sweden. Vol. 17: 396. 1950.
4. Fenner, H., and Archibald, J. G. A Critical Study of Energy Determination in Fresh and Dried Cow Feces. Paper Submitted to J. Dairy Sci.
5. Flatt, W. P. Effect of Methods of Preservation and Storage of Fecal Samples on Energy Losses. Paper presented at the Ann. Meeting of the A.D.S.A., June 26-29, 1957 at Oklahoma A. & M. College, Stillwater, Oklahoma.
6. Kleiber, Max. The California Apparatus for Respiration Trials With Large Animals. Hilgardia. Vol. 9: Jan. 1935. No. 1.
7. Swift, Raymond W., and French, Cyrus E. Energy Metabolism and Nutrition. The Scarecrow Press. Washington, D. C. 1954.
8. Snedecor, G. W. Statistical Methods. 5th ed. Iowa State College Press, Ames, Iowa. 1956.

Table I - Highest Ratio of Sample to Primer that Burned Completely

| Material       | Dry Matter | Cal./gm.<br>wet material | Ratio by wt.<br>Dimethylformamide | Material/Primer<br>Butyl Cellosolve |
|----------------|------------|--------------------------|-----------------------------------|-------------------------------------|
| Corn Silage    | 31%        | 1452                     | 10.5                              | 12.0                                |
| Alfalfa Silage | 24%        | 1245                     | 4.6                               | 6.3                                 |
| Feces          | 20%        | 890                      | 3.0                               | 4.0                                 |
| Urine          | 5%         | 150                      | 0.5                               | 1.0                                 |

Table II - Composition of Burning Mixtures (from Table I)

| Material       | Dimethylformamide Mixtures |   | Butyl Cellosolve Mixtures |   |
|----------------|----------------------------|---|---------------------------|---|
|                | % H <sub>2</sub> O         | Ratio of Total/<br>calories gms. H <sub>2</sub> O | % H <sub>2</sub> O        | Ratio of Total/<br>calories gms. H <sub>2</sub> O |
| Corn Silage    | 64                         | 2934  | 65                        | 2993  |
| Alfalfa Silage | 62                         | 3447  | 66                        | 3245  |
| Feces          | 60                         | 3743  | 65                        | 3467  |
| Urine          | 32                         | 13471   | 48                        | 8265  |

Table III - Linear Regression Data

| Material       | Primer                 | Number of<br>determinations | Slope (b)<br>cal/ratio unit | Standard error<br>of regression coeff. |
|----------------|------------------------|-----------------------------|-----------------------------|--|
| Corn Silage    | Butyl Cello-<br>solve  | 8                           | -2.97*                      | ±1.15                                  |
| " "            | Dimethyl-<br>formamide | 16                          | -5.56*                      | ±2.18                                  |
| Alfalfa Silage | Butyl Cello-<br>solve  | 7                           | -12.99**                    | ±2.47                                  |
| " "            | Dimethyl-<br>formamide | 7                           | -4.50                       | ±34.0                                  |

\* Significant at the 0.05 level of probability.

\*\* Significant at the 0.01 level of probability.